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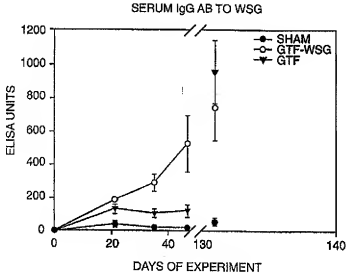

(57) Abstract

The present invention provides glucan-based compositions and methods for stimulating an immune response against mutans streptococci components and vaccines and methods for the treatment and prevention of dental caries. In a preferred embodiment, a glucan polymer is covalently bound to one or more T cell-dependent antigens to form a conjugate vaccine. The T cell-dependent antigen preferably contains epitopes of one or more mutans streptococcal proteins, such as a glucosyltransferase. Moreover, one or more moieties, including haptens, may be conjugated to the glucan or to the glucan-T cell-dependent composition. In a preferred embodiment, these moieties are peptides which contain immunogenic epitopes corresponding to components of a mutants streptococcus.

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Conjugate Vaccines for the Prevention of Dental Caries

GOVERNMENT INTEREST

This invention was made with Government support under NIH grant no. DE-04733 awarded by the National Institutes of Health. The U.S. Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to the methods of increasing the immunogenicity of glucan epitopes and associated components, preferably, by the preparation of conjugate vaccines comprised of glucan polysaccharides and T cell-dependent antigens, preferably derived from cariogenic Streptococci. In a preferred embodiment, the T cell-dependent antigen is a mutans streptococcal glucose binding protein or glucosyltransferase, fragment, peptide, or combination thereof. The immunogenic compositions of the present invention may be useful in the prevention of dental caries.

BACKGROUND

Dental caries, or tooth decay, results from the erosion of mineral in the enamel and underlying dentin layers of the tooth by the lactic acid secreted by a discrete class of streptococcal bacteria. These cariogenic bacteria, collectively called "mutans streptococci" have been genetically classified into at least four distinct species: *Streptococcus mutans*, *S. rattus*, *S. cricetus*, and *S. sobrinus*. Of these, *S. mutans*, and, to a lesser extent, *S. sobrinus*, are common human pathogens. The biology and cariogenic potential of these organisms has been reviewed by A.L. Coykendall and K.B. Gustafson, *Taxonomy of Streptococcus mutans, in* Molecular Microbiology and

flora, primarily the non-cariogenic *Streptococcus salivarius* and *Streptococcus mitis*, which colonize soft epithelial surfaces. Interestingly, the eruption of primary teeth does not result in the immediate colonization of cariogenic streptococci. Rather, and for reasons that are not entirely understood, newly erupted dental surfaces do not usually support the attachment of mutans, but are often colonized by noncariogenic *S. sanguis*. Subsequently, however, oral colonization with mutans streptococci occurs between about eighteen and thirty-six months of age. Although this "window of infectivity" between tooth eruption and mutans colonization remains a poorly understood phenomena, it nevertheless provides a potential opportunity to block mutans invasion before it starts.

Like most infections, mutans streptococcal infections elicit antibody responses in the host, and mounting evidence suggests that a healthy immune system is critical to oral health. Indeed, a low incidence of dental caries has been correlated with high levels of IgG antibodies to mutans surface proteins. Although IgG is usually not considered a secreted protein, antibodies of this isotype may access mutans streptococci at the gumline, through the gingival crevice. Moreover, anti-mutans IgA antibodies, secreted directly into the salivary milieu, appear to block bacterial attachment and plaque formation.

Mutans streptococcal infection is arguably the most common bacterial disease in humans. Moreover, the tooth decay generated by these bacteria represent the principal cause of tooth loss among adults below the age of forty. A properly directed vaccine could reduce the incidence of caries in infected adults. In addition, because children are immunocompetent by this age (Smith and Taubman, Crit. Rev. Oral Biol. Med. 4(3/4):335-41 (1993)), early vaccination could even prevent mutans colonization entirely, potentially resulting in a caries-free mouth.

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Thus, the possibility of controlling this caries by active immunization is currently under intensive investigation. The various strategies for creating a prophylactic caries vaccine are reviewed in Immunologic Aspects of Dental Caries: Selection of Immunogens for a Caries Vaccine and Cross Reactivity of Antisera to Oral Microorganisms with Mammalian Tissues (W. Bowen, R. Genco & T. O'Brien eds. 1976) Information Retrieval Inc.; and D.J. Smith and M.A. Taubman, *Vaccines Against Dental Caries Infection in* New Generation Vaccines (M.M. Levine, G.C. Woodrow, J.B. Kaper, & G.S. Cobon eds., 2d ed. 1997), Marcel Dekker, Inc., (each of which are incorporated herein by reference). These attempts range from oral ingestion of highly cariogenic strains of whole, killed S. mutans bacteria (Michalek *et al.*, Science 192:1238-40 (1996)), to parenteral vaccines using peptides from critical regions of GTF or AgI/II proteins. None of these vaccines has, by themselves, proved to be a panacea against cariogenic infection.

Thus, there remains a need in the art for a safe and efficacious vaccine against mutans streptococci.

SUMMARY OF THE INVENTION

The present invention addresses these needs by providing novel vaccines based on the extracellular glucan component of the cariogenic plaque. Although the glucan may be administered alone, it is preferred that the antigenicity of the glucan be enhanced, preferably, by covalently coupling the glucan to one or more moieties, preferably, T cell-dependent antigens, to form a conjugate vaccine. In a preferred embodiment, the TD antigen contains epitopes of one or more mutans streptococci proteins, such as AgI/II or a GBP, and preferably, epitopes from a GTF.

In addition, one or more additional moieties, including haptens, may be conjugated to the glucan or to the glucan–TD composition. In a preferred embodiment, these moieties are peptides that contain immunogenic epitopes

corresponding to mutans streptococcal components. Preferably, antibodies generated against these epitopes bind to an AgI/II bacterial adhesion protein, or to a GBP, more preferably, to a GTF, and most preferably, to the catalytic or glucan binding site of a GTF.

The present invention thus provides compositions and methods for stimulating an immune response against mutans streptococci components, including glucans and other extracellular or cell associated components, and vaccines and methods for the treatment and prevention of dental caries.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates levels of Serum IgG antibodies induced by vaccination with the GTF-WSG conjugate as compared to GTF alone or PBS (sham) as of 21, 35, 47, and 131 days after the primary innoculation.

Figure 2 illustrates the percent inhibition of WIG (panel 2A) and WSG (panel 2B) polymerization activity by sera from rats immunized with GTF-WSG versus GTF alone or PBS (sham).

Figure 3 illustrates the relative levels of anti-WSG IgA at day 21 (panel 3A) and day 101 (panel 3B) in the saliva of rats immunized with the GTF-WSG conjugate as compared to GTF or WSG alone, or PBS (sham).

Figure 4 illustrates the relative levels of salivary anti-GTF IgA 21, 35, and 101 days after primary inoculation (panels 4A, 4B, and 4C, respectively) with PBS (sham), WSG, GTF-WSG, or GTF.

DETAILED DESCRIPTION OF THE INVENTION

The etiology of dental caries is associated with the acid by-products of bacterial metabolism. The production of these by-products is related to a group of aciduric oral microorganisms collectively referred to as the mutans streptococci. Important microorganisms in this group that are found in

humans include *Streptococcus mutans* and *S. sobrinus*. Loesche, Microbiol. Rev. 50: 353-80 (1986).

The infection process and subsequent pathological consequences of mutans colonization occur in a milieu that is perfused with elements of the mucosal and systemic immune systems. In light of this, many immunization strategies have been explored in an attempt to induce immunity to relevant mutans streptococcal virulence components that could ultimately protect the host from dental caries.

Many of these strategies demonstrated a degree of protection in the experimental dental caries caused by infection of a susceptible rat or primate animal model with cariogenic mutans streptococci. Both the active and passive routes have been employed to immunize with mutans streptococci, and isolated mutans components, including those associated with initial attachments ((e.g., adhesin Ag I/II) (Russell *et al.*, Infect. Immun. 28:486-43 (1980); and Ma *et al.*, Clin. Exp. Immunol. 77:331-37 (1989)), or with subsequent accumulation ((e.g., GTF) (Taubman *et al.*, J. Immunol. 118:710-20 (1977)).

A significant feature of the molecular pathogenesis of dental caries appears to be the role of accumulation of these, and/or related mutans streptococci, in dental plaque. The principle framework of plaque is provided by high molecular weight, branched, glucose polymers called glucans. Glucans are synthesized from sucrose by bacterially secreted glucosyltransferase (GTF) enzymes. Asakawa *et al.*, J. Gen. Microbiol. 132:2873-83 (1988) and Hamada *et al.*, Microbiol. Rev. 44:331-84 (1980).

The glucans of various mutans streptococcal strains contain varying ratios of α-1,3 and α-1,6 linked glucosidic linkages. Generally, these molecules contain long runs of α-1,3 linkages and shorter runs of α-1,6 linkages, with frequent branching points in which the same glucosyl residue is bridged to chains of α-1,3 and α-1,6-linked runs. These molecules are

generally classified as water soluble (WSG) and water insoluble glucans (WIG). The degree of water solubility and association of the glucans with the cell surface of the mutans streptococci appears to be a function of the relative predominance of linkage types and of branches. The predominant WSG class is rich in α-1,6 linkages, whereas WIG has more α-1,3 linkages and is more densely branched. In contrast, dextran, the extracellular polysaccharide of various *Leuconostoc* species, is a virtually pure α-1,6 linked linear glucose polymer.

Interference with the synthesis or accumulation of steptoccoccal glucans is an opportune target for intervention because the formation of these extracellular polysaccharides is absolutely critical to the cariogenic plaque and resultant caries. Indeed, immunization with GTF, or GTF peptide antigens, may result in protection from experimental dental caries in rodent models, presumably, by preventing the synthesis of extracellular glucans. Taubman *et al.*, J. Immunol. 118:710-20 (1977); Smith *et al.*, Infect. Immun. 37:656-61 (1982); and Smith *et al.*, Infect. Immun. 26:82-89 (1987). In humans, such immunization results in the induction of salivary IgA antibody (SIgA), accompanied by interference with reaccumulation of indigenous mutans streptococci after dental prophylaxis. Smith *et al.*, Infect. Immun. 55:2562-69 (1987). Local injection, gastric intubation, oral administration and topical application have each demonstrated some protective effect using these antigens. Although the exact basis for experimental protection with such GTF-type vaccines is presently unknown, it appears likely that such protection can involve functional inhibition of the catalytic and/or the glucan binding activity of GTF.

Notably, none of these strategies are designed to elicit an antigenic response against the principle structural components of the cariogenic plaque. A single prior study suggested that administration of *S. sobrinus* GTF, which was noncovalently bound to particles of water-insoluble glucan,

appeared to elicit a greater secretory immune response than did immunization with soluble GTF. J.L. Ebersole, M.A. Taubman, D.J. Smith, *Adjuvants, Glucosyltransferase and Caries Vaccine, in* Proceedings 'Glucosyltransferase, Glucan, Sucrose, and Dental Caries' (R. Doyle & J.E. Ciardi eds.), Sp. Supp. Chemical Senses, pp. 241-48 (1983). The focus of this study was to elicit antibodies against GTF. Ebersole and coworkers tested GTF combined with $Al(OH)_3$, Freund's and muramyl dipeptide. Along with these known adjuvants, particulate glucan was apparently tested as a depot-type adjuvant, similar to $Al(OH)_3$. Consequently, the presence or absence of an anti-glucan response would have been irrelevant to this study and, indeed, the investigators made no attempt to measure an anti-glucan response.

In contrast to the approaches taken in the prior art, the novel immunogenic compositions and vaccines of the present invention are designed to elicit antibodies against streptococcal glucans themselves. These antibodies provide protection against cariogenic lesions. Although the inventors do not wish to be bound to any particular theory underlying this effect, various scenarios are possible. For example, an immune response to glucan may intercept bacterial aggregation, thereby preventing caries, in several ways. Possibly, anti-glucan antibodies could aggregate and clear mutans streptococci from the oral cavity via cell-bound glucan, or interfere with the binding of glucan to the various non-catalytic glucose binding proteins (GBPs) that contribute to plaque stability. Alternatively, or in addition, anti-glucan antibodies could alter or abrogate the glucan chain lengthening process, by interfering with GTF catalytic activity, or by inhibiting the binding of glucan to GTFs.

The use of glucan as a component of a dental caries vaccine is complicated by the fact that these simple polysaccharides are type 2 T-independent (TI-2) antigens. The TI-2 class of antigens are predominantly

linear antigens that are not readily degraded in the body and that have regularly spaced, highly repeating determinants, as reviewed in Mond et al., Ann. Rev. Immun. 14:655-92 (1995). TI-2 antigens commonly comprise large polysaccharide polymers such as those derived from bacterial cell walls or flagella. Other common examples of TI-2 antigens include FICOLL, D-amino acid polymers, polyvinylpyrrolidone, and some highly repetitive polypeptides. When a TI-2 antigen encounters a B cell that expresses cognate cell surface receptors, the antigen binds to multiple B cell surface receptors but is not internalized. A TI-2 antigen generally remains, unprocessed, on the cell surface and stimulates the T-cell independent pathway directly, without direct T cell intervention. Not only do most TI-2 antigen molecules remain intact on the surface of macrophage and B cells, but even if internalized, these antigens are not degraded by endosomal proteases, nor do they efficiently bind to MHC molecules; consequently, they cannot themselves enter the highly productive Class II (T cell-dependent) pathway. Thus, TI-2 antigens induce small primary, and essentially no secondary immune responses. This meager response generally provides little, if any, immunoprotective effect.

Moreover, responses to TI-2 antigens are extremely poor in children less than two years of age. This could suggest against the use of polysaccharide antigens for the twelve to eighteen month old population targeted for a dental caries vaccine. Because children are not generally infected with mutans streptococci prior to about eighteen months of age, vaccination of young children is a particularly preferred method of prophylaxis. Smith & Taubman, Crit. Rev. Oral Biol. Med. 4(3/4):335-41 (1993).

However, conjugating protein or TD peptides to poorly immunogenic TI-2 antigens, significantly improves the induction of immunity to the TI-2 antigen and has resulted in the design of polysaccharide-protein conjugate

vaccines now in common use. Robbins and Schneerson, J. Infect. Dis. 161:821-32 (1990); reviewed in *Conjugate Vaccines, in* Contrib. Microbiol. Immunol. Vol. 10, (J.M. Cruse & R.E. Lewis Jr. eds., 1989). For example, diphtheria toxoid has been conjugated to the capsular polysaccharide of *Hemophihus influenzae* (HibDT) and is licensed for administration to children younger than eighteen months old, and Hib vaccinations are reccommended as early as 2 months of age. Physicians' Desk Reference, pages 1162-1163 (1994); and Recommended Childhood Immunization Schedule United States, for January - June 1996.

Moreover, it has recently been shown that conjugation of a T-dependent antigen to a poorly immunogenic polysaccharide can enhance the immunogenic response to both the T-dependent and T-independent components. In addition, the antibody response to haptens (including non-TD peptides) can also be dramatically enhanced if further conjugated to the T-dependent or T-independent carrier, or both. Lees *et al.*, Vaccine 1160-66 (1994); U.S. Patent No. 5,585,100 (Mond and Lees); and U.S. Patent Appln. No. 08/468,359, filed June 6, 1995 (Mond and Lees), incorporated herein by reference. Haptens are commonly defined as small molecules that are very poorly immunogenic themselves. However, a hapten contains at least one B cell epitope, and thus can be recognized by pre-formed antibodies.

Thus, in one embodiment a high molecular weight TI-2 antigen, such as a bacterial polysaccharide, is covalently bound to at least one TD antigen to form a dual conjugate (defined in Lees *et al.*, Vaccine 1160-66 (1994); U.S. Patent No. 5,585,100 (Mond and Lees); and U.S. Patent Appln. No. 08/468,359, filed June 6, 1995 (Mond and Lees). In this embodiment, antibodies raised against that antigen, or to a hapten bound to the conjugate, bind to at least one component of a streptococcal mutans, preferably, a GBP or mutans surface antigen, more preferably, a GTF.

Moreover, the present invention preferably comprises at least one streptococcal glucan, and at least one T-dependent carrier molecule covalently conjugated to the glucan. As a result of the contributions of both types the TI-2 and TD carriers, the immunogenic constructs of the invention are extremely potent activators of T cell help via mechanisms such as enhanced antigen presentation by B cells, macrophages or other antigen presenting cells. Such a construct can elicit immunological memory and result in long lived antibody formation against the glucan component in adults and children.

The glucan component of the invention may be derived from any source, including synthetic, and other bacterial polysaccharides, such as *Leuconostoc* dextrans may also be used. However, it is preferred that the glucans have a structure similar to that synthesized by cariogenic streptococcal mutans, preferably, *S. sobrinus*, most preferably, *S. mutans*. The GTFs of a streptococcal mutan each produce a different form of glucan, broadly classified as WSG (water soluble glucans) or WIG (water insoluble glucans). WSG glucans are preferred. Methods of purifying glucans are well known in the art. Smith *et al.*, Infect. Immun. 61:2899-2905 (1993); Taubman *et al.*, J. Oral. Pathol. 17:466-70 (1988); and Taubman *et al.*, Infect. Immun. 63:3088-93 (1995).

As to the size of the glucans of the invention, it has been suggested that low-molecular weight polysaccharides may inhibit immunogenicity. Dintzis *et al.*, J. Immunol. 143:1239-44 (1989); Dintzis *et al.*, Fed. Am. Soc. Exp. Biol. 46(3):777 Abstract (1987); Dintzis and Dintzis, Proc. Natl. Acad. Sci. USA 89:1113-17 (1992); Symer *et al.*, J. Immunol. 155:5608-16 (1995); Reim *et al.*, Mol. Immunol. 33:1377-88 (1996); Watson *et al.*, J. Immunol. 156:2443-50 (1996); and Dintzis *et al.*, U.S. Pat. No's. 5,370,871 and 5,126,131. Moreover, the removal of low molecular weight carbohydrate components may enhance the immunogenicity of the TD components of dual

conjugate vaccines. Lees *et al.*, Vaccine 1160-66 (1994); U.S. Patent No. 5,585,100 (Mond and Lees); and U.S. Patent Appln. No. 08/468,359, filed June 6, 1995 (Mond and Lees). Therefore, the immunogenic compositions of the invention may be purified to remove low molecular weight polysaccharides of less than 100 kDa, 250 kDa, 500 kDa, 750 kDa, 1000 kDa, or 2000 kDa molecular mass. Such purification may be accomplished by gel filtration or any other of the number of techniqes well known in the art.

The glucan antigen may be administered directly, or bound to a moiety. The moiety may be any molecule other than the glucan, preferably, a TD antigen, or a hapten. However, because TI-2 antigens such as glucan are poorly immunogenic themselves, it is highly preferred that steps be taken to increase the immunogenicity of glucan epitopes. Thus, in the preferred embodiment, the glucan is associated with, and preferably, covalently bound to at least one TD antigen, forming a dual conjugate composition comprising a TI-2 (glucan) carrier and at least one TD carrier. Additional moieties, including other TD antigens, may be further conjugated to this dual carrier construct.

TD antigens are well known in the art and include, for example, serum albumins, Keyhole Limpet hemocyanin, *E. coli* LT, Horseshoe crab hemocyanin, cholera toxin and toxoid, diphtheria toxoid, pertussis toxoid, tetanus toxoid, and bacterial outermembrane proteins. Some additional TD antigens that may be used in the present invention are described in W.E. Dick and M. Beurret, *Conjugate Vaccines, in* Contrib. Microbiol. Immunol. Vol. 10, pp. 48-114, (J.M. Cruse & R.E. Lewis Jr. eds., 1989), incorporated herein by reference.

In a preferred embodiment, at least one moiety is a TD component derived from a Streptococcus, preferably a cariogenic mutans streptococci, preferably, *S. sobrinus*, most preferably, *S. mutans*. A TD antigen is defined

as a molecule, generally a protein or peptide, which contains both T and B cell epitopes, and thus elicits a T cell-dependent response.

TD antigens may be identified as containing both T and B cell epitopes according to the procedure of Lett *et al.*, Infect. and Immun 62(3):785-92 (1994), or by any other technique known in the art, including the use of algorithms. Algorithms to predict features associated with T and B epitopes from amino acid sequence data are described in Garnier *et al.*, J. Mol. Biol. 120:97-120 (1978); Hopp *et al.*, Proc. Natl. Acad. Sci. 78 (1981); Rothbard *et al.*, EMBO J. 7:93-100 (1988); and Berzofsky *et al.*, Immunol. Rev. 98:9-52 (1987). Peptides predicted to contain both T and B epitopes can be purified or synthesized and tested for the ability to elicit a T cell-dependent response, for example, by immunizing with the peptide and observing class switching and memory response. Standard techniques for immunization and analysis of the subsequent antibody response are found in Antibodies: A Laboratory Manual, (Harlow & Lane eds., 1988), Cold Spring Harbor Laboratory Press, (incorporated herein by reference).

In one embodiment, the TD antigen is an adhesin, or fragment thereof, for example, the 42 kDa AgII fragment used by Hajishengallis *et al.*, J. Immunol. 154:4322-32 (1995). In a preferred embodiment, the TD component is a GTF, preferably a GTF from a cariogenic streptococcal mutans, more preferably, *S. sobrinus*, and most preferably, *S. mutans*. The term GTF encompasses the naturally occurring, full length amino acid sequence of a glucosyltransferease, as well as any peptide, fusion protein, or fragment thereof containing at least one T- and at least one B-cell dependent epitope. The GTF may be purified from bacteria, produced recombinantly, engineered as a recombinant fusion protein, or synthesized synthetically. Methods for purification of GTF are incorporated herein by U.S. Patent Nos. 4,250262 and 4,438,200 (Taubman *et al.*); and Smith *et al.*, Infect. Immun. 23:446-52 (1979).

The production and expression of recombinant proteins and fusion proteins is well known in the art and can be carried out using conventional procedures, such as those in Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Vols. 1-3, (2d ed. 1989), Cold Spring Harbor Laboratory Press (incorporated herein by reference). GTF or other mutans-specific fusion proteins can also be designed by fusing sequences encoding mutans polypeptides to sequences encoding another polypeptide to aid in the purification of the mutans-specific epitopes. An example of such a fusion is a fusion of sequences encoding a GTF polypeptide to sequences encoding the product of the malE gene of the pMAL-c2 vector of New England Biolabs, Inc., or to a hexahistidine sequence. Such fusions allow for affinity purification of the fusion protein. In addition, methods for removing the non-mutans sequences from the fusion protein after purification are well known in the art. Fusion proteins may also be designed to enhance the immunogenicity of mutans epitopes, for example, by fusing a mutans streptococcal polypeptide sequence to a strong TD antigen such as cholera toxin B subunit. Dertzbaugh *et al.*, Infect. Immun. 58:70-79 (1990).

The moiety may also be a fragment or peptide of a mutans streptococcal protein, preferably, a TD antigen. For example, a GTF may be isolated and purified according to standard methods, and subject to chemical fragmentation. For example, the isolated and purified GTF polypeptide can be treated with cyanogen bromide under conventional conditions that result in fragmentation of the GTF polypeptide by specific hydrolysis on the carboxyl side of the methionine residues within the GTF polypeptide. Gross, Methods in Enz. 11:238-255 (1967). Chemical fragmentation includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues. Gross, Methods in Enz. 11:238-255, (1967). This can further include additional steps, such as a carboxymethylation step to convert cysteine residues to an unreactive

species. It is understood of course that many chemicals could be used to fragment mutans polypeptides and that this embodiment in no way limits the scope of the invention.

Alternatively, immunogenic mutans peptides can be generated using enzymes that cleave the polypeptide at specific amino acid residues. For example, an isolated and purified GTF polypeptide can be treated with Achromobacter protease I under conventional conditions that result in fragmentation of the GTF polypeptide by specific hydrolysis on the carboxyl side of the lysine residues within the GTF polypeptide. Masaki *et al.*, Biochim. Biophys. Acta 660:44-50 (1981); Masaki *et al.*, Biochim. Biophys. Acta 660:51-55 (1981). Enzymatic fragmentation includes the use of a protease such as Asparaginylendopeptidase, Arginylendopeptidase, *Achrombobacter* protease I, Trypsin, *Staphlococcus aureus* V8 protease, Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to result in cleavage at specific amino acid residues. Sakiyama and Nakat, U.S. Patent No. 5,248,599; Masaki *et al.*, Biochim. Biophys. Acta 660:44-50 (1981); Masaki *et al.*, Biochim. Biophys. Acta 660:51-55 (1981); and Cleveland, J. Biol. Chem. 3:1102-06 (1977), which are hereby incorporated by reference. Other enzymatic and chemical treatments can likewise be used to specifically fragment GTF or other streptococcal mutans polypeptides.

Synthetic GTF polypeptides and peptides can be generated by a variety of conventional techniques using published GTF sequences, e.g., Akoi *et al.*, Infect Immun 53:587-94 (1986); Banas *et al.*, Infect Immun., 58:667-73 (1990); Hanada and Kuramitsu, Infect. Immun 57:2079-85 (1989); Ferretti *et al.*, Infect. Immun. 56:1585-88 (1988); Russell *et al.*, J. Dental Res., 67:543-47 (1988); Ueda *et al.*, Gene, 69:1101-09 (1988), each of which is incorporated by reference. Such techniques include those described in Merrifield, Methods Enzymol. 289:3-13 (1997); Ball and Mascagni, Int. J.

Pept. Protein Res. 48:31-47 (1996); Molina *et al.*, Pept. Res. 9:151-155 (1996); Fox, Mol. Biotechnol. 3:249-258 (1995); and Lepage *et al.*, Anal. Biochem. 213: 40-48 (1993), incorporated herein by reference.

In another embodiment, one or more moieties may be directly or indirectly covalently conjugated to the glucan, or to either or both components of the glucan--TD antigen composition. These moieties may be haptens, TI-2 or TD antigens, and are preferably proteins or peptides.

In a preferred embodiment, the moieties conjugated to the glucan, or to either or both components of the glucan--TD antigen composition are peptides which contain immunogenic epitopes corresponding to components of a mutans streptococci. In one embodiment, antibodies generated against these epitopes bind to an AgI/II bacterial adhesion protein, preferably, to a region implicated in AgI/II binding, for example, the saliva-binding region of Toida *et al.*, Infect Immun. 65(3):909-15 (1997). In another embodiment, antibodies generated against the immunogenic epitopes bind to a glucan binding protein (GBP) (Smith and Taubman, Infect. and Immun. 64(8):3069-73 (1996), incorporated by reference), more preferably, to a GTF, most preferably, to the catalytic or glucan binding site of a GTF. In a preferred embodiment, at least two different peptides are directly or indirectly conjugated to the glucan carrier. In one embodiment, multiple copies of at least one mutans peptide are conjugated to a core matrix, which is then directly, or indirectly, bound to the glucan carrier. Taubman *et al.*, U.S. Patent No. 5,686,075, incorporated by reference.

Examples of acceptable peptides are those directed against *S. mutans* surface protein antigen, as described by Takahashi and coworkers (J. Immunol. 146:332-36 (1991)) and the peptide GAVDSILGGVATYGA (SEQ ID NO:16) of Lehner *et al.*, J. Immunol. 143:2699-705 (1989). Preferred peptides are those containing epitopes of an AgI/II protein, including, YEKEPTTPPTRTPDQ (SEQ ID NO:17), TPEDPTDPTDPQDPSS

(SEQ ID NO:18), and ANAANEADYQAKLTAYQTEC (SEQ ID NO:19). Lett *et al.*, Infect. and Immun 62(3):785-92 (1994); Lett *et al.*, Infect. and Immun. 63(7):2645-51 (1995); and Takahashi and coworkers (J. Immunol. 146:332-36 (1991), each of which is incorporated by reference.

More preferred peptides are those which contain antigenic epitopes of a GTF. Examples of such peptides have been described by Chia *et al.*, Infect. Immun. 65(3):1126-30, (1997); Smith *et al.*, Infect Immun. 61:2899-905 (1993); Chia *et al.*, Infect. Immun. 61:4689-95 (1993); Lett *et al.*, Infect. Immun. 62:785-92 (1994); Dertzbaugh *et al.*, Infect Immun. 58:70-79 (1990); Chia *et al.*, Infect. Immun. 61:1563-66 (1993); and U.S. Patents 5,686,070 and 4,150,116 (Taubman *et al.*) each of which is incorporated herein by reference. These peptides are represented by:

a)	DGKLRYYDANSGDQAFNKSV	SEQ ID NO:1;
b)	PLDKRSGLNPLIHNSLVDREVDDRE	SEQ ID NO:2;
c)	TGAQTIKGQKLYFKANGQQVKG	SEQ ID NO:3;
d)	QWNGESEKPYDDHL	SEQ ID NO:4;
e)	GGYEFLLANDVDNSNPVVQ	SEQ ID NO:5;
f)	ANDVDNSNPVVQAEQLNWL	SEQ ID NO:6;
g)	GGYEFLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:7;
h)	GGYEDLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:8;
i)	GGYEFLLANDVDNSNPIVQAEQLNWL	SEQ ID NO:9;
j)	AGYELLLANDVDNSNPVVQAEQLNHL	SEQ ID NO:10;
k)	DANFDSIRVDAVDNVDADVVQIA	SEQ ID NO:11;
l)	DANFDSIRVDAEDNVDADQLQIS	SEQ ID NO:12;
m)	DSIRVDAVD	SEQ ID NO:13;
n)	YEKEPTPPTRTPDQ	SEQ ID NO:14; and
o)	SAWNSDSEKPFDDHL	SEQ ID NO:15.

In the present invention, moieties, including TD antigens and haptens, are conjugated to the glucan carrier. This association may be direct, or indirect, for example, through a linker, a TD antigen, or through any other moiety. Any form of chemical binding, including covalent, is within the scope of this invention. Covalent binding is preferred. Methods of conjugation are well known to those of ordinary skill in the art, and include the heteroligation

techniques of Brunswick *et al.*, J. Immunol., 140:3364 (1988); S.S. Wong, Chemistry of Protein Conjugates and Crosslinking, (1991), CRC Press, Boston; Brenkeley *et al.*, *Brief Survey of Methods for Preparing Protein Conjugates With Dyes, Haptens and Cross-Linking Agents, in* Bioconjugate Chemistry, 3, No. 1 (Jan. 1992); and G.T. Hermanson, Bioconjugate Techniques, (1996), Academic Press, San Diego, each of which are specifically incorporated by reference.

A particularly preferred method of covalent conjugation is via CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate) activation of the polysaccharide, set forth in application Serial Nos. 08/482,616 and 08/482,666, filed June 7, 1995, (08/482,616 being now abandoned), which are a continuation-in-part applications of application Serial No. 08/408,717, filed March 22, 1995, and issued July 29, 1997, as U.S. Patent No. 5,651,971, and which is a continuation-in-part of application 08/124,491, filed September 22, 1993, now abandoned, and further set forth in the continuation of application 08/408,717, application 08/456,694, filed June 1, 1995, which issued December 2, 1997 as U.S. Patent No. 5,693,326, and as further set forth in the continuation-in-part of application 08/124,491, filed September 22, 1993, the disclosures of which are all specifically incorporated herein by reference. The proteins and polysaccharides may be coupled, either directly or indirectly, using a spacer, for example, using homobifunctional or heterobifunctional vinylsulfones as described in U.S. Provisional Patent Appln. No. 60/017,103 filed on May 9, 1996, and U.S. Patent Appln. No. 08/852,733 filed on May 7, 1997, each in the name of Andrew Lees. The protein and/or the polysaccharide can be derivatized or functionalized prior to the conjugation reaction procedure (e.g., with thiols, amines, or hydrazides). Other suitable protein/polysaccharide conjugation techniques for use with this invention include protein/polysaccharide coupling using uronium salts and haloacyl reagents as described in U.S. Provisional

Patent Appln. Nos. 60/041,781 (filed March 24, 1997) and 60/042,379 (filed April 24, 1997). All of the above-referenced patents and patent applications are entirely incorporated herein by reference.

The process of synthesizing the construct of the invention allows one to advantageously control the physical and chemical properties of the final product. The properties that may be controlled include modifying the charge on primary and secondary carriers (an advantage in light of evidence that cationized proteins may be more immunogenic), varying the size of the construct by varying the size of the TI-2 carriers, selecting the degree of crosslinking of the construct (to obtain variations of size and half-life in the circulation), selecting the number of copies of secondary carriers conjugated to TD carriers, and targeting to selected cell populations (such as to macrophages to enhance antigen presentation).

The immune response to the construct of the invention may be further enhanced by the addition of immunomodulators and/or cell targeting moieties. These entities are co-administered, and preferably chemically conjugated to the immunogenic composition and include, for example, (1) detoxified lipopolysaccharides or derivatives, (2) muramyl dipeptides, (3) carbohydrates, lipids, and peptides that may interact with cell surface determinants to target the construct to immunologically relevant cells, (4) interleukins, and (5) antibodies that may interact with cell surface components. In a preferred embodiment the immunogenicity of the construct may be enhanced by the co-administration or conjugation of an adjuvanting lipoprotein, as described in the copending application, incorporated herein by reference: *Induction and Enhancement of the Immune Response to Type 2 T Cell-independent Antigens Conjugated to Lipid or Lipid-containing Moieties* of Mond and Snapper, filed March 16, 1998 (Serial No. unassigned). Lipoproteins are preferably conjugated to the glucan, TD

component, or both, by the methods described in U.S. Patent No. 5,693,326 to Lees (incorporated herein by reference).

The glucan conjugates of the invention stimulate the immune system to produce anti-mutans antibodies which intercept the GTF-glucan-mediated virulence pathway. Administration of this conjugate to a patient will increase the immunogenicity of the glucan and of any mutans peptide component, resulting in elevated levels of antibody to both peptide and carbohydrate components of the vaccine. The resultant antibody titers will protect against infection with cariogenic mutans streptococci. The degree of protection may be assayed in any of the animal models known in the art, such as the rodent caries model described in Taubman and Smith, J. Immun. 118(2):710-20 (1977) (incorporated herein by reference), but is equally applicable to patients.

The invention also relates to the treatment of a patient by administration of an immunostimulatory amount of the vaccine. A patient is hereby defined as any person or non-human animal in need of immune stimulation, or to any subject for whom treatment may be beneficial, including humans, and non-human animals. Such non-human animals to be treated include all domesticated and feral vertebrates, preferably but are not limited to mice, rats, rabbits, hamsters, dogs, cats, swine, sheep, horses, cattle, and non-human primates. One of skill in the art will, of course, recognize that the choice of glucan and non-polysaccharide antigens will depend on the streptococcal mutans species or subtypes to be vaccinated against in a particular system. An immunostimulatory amount refers to that amount of vaccine that is able to stimulate the production of antibodies directed against a mutans streptococcal epitope. Preferably, an immumostimulatory amount refers to that amount of vaccine that is able to stimulate an immune response in a patient which is sufficient to prevent, ameliorate, or otherwise treat dental caries.

Secondary booster immunizations may be given at intervals ranging from one week to many months later. The dosage of the primary and secondary inocula can be readily determined by those of ordinary skill in the art, but an acceptable range is 0.01 µg to 100 µg per inoculum. The amount to be administered and the frequency of administration can be determined empirically and will take into consideration the age and size of the patient being treated and the stage of the dental caries disease (e.g., prior to colonization with mutans streptococci, early in the colonization process, or after carious lesions are detected). In a highly preferred embodiment, the patient is vaccinated after the immune system has become competent to respond to the composition, but before the mouth is fully colonized by mutans streptococci. In a human patient, this period spans from about eighteen to 36 months of age. Moreover, treatment may begin as early as two months of age.

Treatment comprises administering the immunogenic composition by any method familiar to those of ordinary skill in the art, including intravenous, intraperitoneal, intracorporeal injection, intra-articular, intraventricular, intrathecal, intramuscular, subcutaneous, topically, tonsillar, intranasally, intravaginally, and orally. The preferred methods of administration are intravenous, intramuscular, intranasal, oral, and subcutaneous injections. The composition may also be given locally, such as by injection into the particular area, either intramuscularly or subcutaneously.

Administration may be parenteral or local, for example by topical application to the minor salivary glands or injection into the gingiva. In order to increase the amount of mutans-specific IgA antibodies in a patient, it is desirable to promote interaction with gut- or nasal-associated lymphoid tissue (GALT, NALT). Thus, mucosal routes of administration are highly preferred, in particular, oral, gastric, and intranasal administration.

As used herein, a vaccine, or pharmaceutical composition, comprises at least one immunological composition, preferably dissolved or suspended in a pharmaceutically acceptable carrier or vehicle. Any pharmaceutically acceptable carrier can be employed for administration of the composition. Carriers can be sterile liquids, such as water, oils, including petroleum oil, animal oil, vegetable oil, peanut oil, soybean oil, mineral oil, sesame oil, and the like. With intravenous administration, the constructs are preferably water soluble and saline is a preferred carrier. Aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, 18th Edition (A. Gennaro, ed., 1990) Mack Pub., Easton, Pa., incorporated by reference. The immunological composition may also be formulated with solubilizing agents, emulsifiers, stabilizers, flavorants, adjuvants, carriers and other components.

In another embodiment of this invention, antibodies specific for mutans streptococcal glucans can be used to detect the presence of glucans in a sample, or for passive immunization, for example, by direct application to the tooth surface. Ma, *et al.*, Clin. Exp. Immunol. 77:331-37 (1989) (incorporated by reference). Monoclonal antibodies are preferred for this application. Any of the compositions of the invention may be used to generate antibodies against mutans streptococci glucans.

The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof such as F(ab')2, and Fab fragments. Antibodies are defined to be specifically binding if they inhibit at least one biological activity of a mutans streptococcus, for example, the binding of at least one glucan binding proteins (e.g. GTFs and GBPs) to the glucan. Alternatively, an antibody specifically binds if it is displaceable in an ELISA or comparable immunological assay. If the antibody is specific for

glucan, for example, antibody binding to glucan can be inhibited by pre-incubation of the antibody with free glucan, as in an ELISA assay. Affinities of antibodies can be readily determined using conventional techniques, for example those described by Scatchard *et al.*, Ann. N.Y Acad. Sci., 51:660 (1949).

Monoclonal antibodies specific for mutans streptococcal glucans can be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, (Kennett, McKearn, & Bechtol eds., 1980), Plenum Press. When used for treating human patients, it is desirable to replace potentially antigenic non-human portions of the antibody with human sequence. A hybrid molecule may be generated in which only the antigen-specific variable, or complementary determining region (CDR) is composed of non-human sequence. These humanized antibodies are thus particularly preferred for clinical use. See, for example, LoBuglio *et al.*, Proc. Natl. Acad. Sci. USA 86:4220-24 (1989); Meredith *et al.*, J. Nucl. Med. 33, 23-29 (1992); Salah *et al.*, Hum. Antibod. Hybridomas 3:19-24 (1992); Knight *et al.*, Mol. Immunol 32:1271-81 (1995); and Lockwood *et al.*, Q.J. Med. 89:903-12, (1996).

Various strategies for designing these humanized antibodies are reviewed in Winter and Milstein, Nature 349:293-99 (1991); Harris, BCSTBS5 23(4):1035-38 (1995); S. Morrison and J. Schlom, Important Advances in Oncology (1990), J.B. Lippincott Co.; L. Presta, *Humanized Monoclonal Antibodies, in* Annual Reports in Medicinal Chemistry (1994) Academic Press; and A. Lewis and J. Crowe, *Generation of Humanized Monoclonal Antibodies by 'Best Fit' Framework Selection and Recombinant Polymerase Chain Reaction", in* Generation of Antibodies by Cell and Gene

Immortalization, Year Immunol. Vol. 7, pp. 110-18 (C. Terhorst, F. Malvasi, & A. Albertini eds., 1993), each of which is incorporated herein by reference.

Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well-known in the art. In general, purified glucan or glucan conjugate is administered to a host animal typically through parenteral injection. The immunogenicity of mutans streptococcal glucans can be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to glucan. Examples of various proceedures and assays useful for the preparation and analysis of polyclonal and monoclonal antibodies are well known in the art and include those described in the series by P. Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology: Practice and Theory of Enzyme Immunoassays, (Burdon & van Knippenberg eds., 3rd ed.,1985) Elsevier, New York; and Antibodies: A Laboratory Manual, (Harlow & Lane eds., 1988), Cold Spring Harbor Laboratory Press; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radio-immunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530, all of which are incorporated by reference.

The present invention is illustrated by the following Examples, which are not intended to be limiting in any way.

Example 1

Preparation of Glucan Conjugates

Preparation of GTFs:

GTFs may be purified as described in Smith *et al.*, Infect. Immun., 55:2562-69 (1987); Smith *et al.*, Infect. Immun. 61:2899-2905 (1993); Taubman *et al.*, J. Oral. Pathol. 17:466-70 (1988); and Taubman *et al.*, Infect. Immun. 63:3088-93 (1995), incorporated by reference. Briefly, *S. sobrinus* 6715 or *S. mutans* SJ32 are grown in glucose-containing defined media, GTFs are isolated from culture media by affinity chromatography on Sephadex G-100 (Pharmacia), with 3 M guanidine HCl as the eluting solvent. GTF-rich eluate is applied to fast-performance liquid chromatography on Superose 6 (Pharmacia) with 3 M guanidine HCl for Elution.

Preparation of Streptococcal Mutans Glucans:

WIG and WSG are prepared as follows: *S. mutans,* or *S. sobrinis* 6715 are grown overnight in defined medium containing sucrose, then centrifuged to remove bacterial cells. The cell-free medium containing GTF activities is neutralized with NaOH and dialyzed overnight at 4°C against PB (20mM phosphate buffer, pH 6.8 , containing 0.2% NaN_3). The dialysate is made 3.1% in sucrose and the reaction is incubated for 2 days at 37°C.

WIG is isolated by from the reaction by centrifugation at 10,000 xg for 20 min. WSG remains in the supernatent. The pelleted WIG fraction is suspended in PB pH 6.8 and dialyzed overnight against the same buffer at 4°C. Protein contaminants are removed from both the WSG supernatent and the WIG dialysate using the phenol extraction technique. Briefly, the material is extracted with an equal volume of water-saturated phenol.

The WIG fraction is then dialyzed extensively against PB.

The WSG fraction is further purified by two, sequential precipitations with 70% ethanol at RT. Following the second ethanol precipitation, the

WSG pellet is dissolved in 6 M guanidine HCl and applied to a Sepharose 6 (Pharmacia) gel filtration column run in 6 M guanidine HCl. Effluent fractions are assayed for carbohydrate using the phenol-sulfuric acid technique of Dubois, et al., Anal. Chem. 28:350-56 (1956), incorporated by reference.

The first detectable carbohydrate peak is taken as WSG. The WSG fractions are pooled and dialyzed extensively against PBS.

Analysis of the WIG and WSG fractions using the BCA assay (Pierce Chemical) indicates that the purified glucans are free of detectable protein.

CDAP Conjugation of TD Antigens to Glucan:

Direct conjugation of a polysaccharide and a protein or peptide using CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate) is essentially as described in Lees, WO 95/08348, incorporated herein by reference. Minor modifications are preferred when the polysaccharide is a glucan and the protein is a glucan-binding protein or a GTF. As a representative example, WSG is suspended in water @ 10 mg/ml on ice, CDAP @ 100 mg/ml in acetonitrile, 2 mg/ml *S. sobrinus* GTF in physiological saline.

50 μl of CDAP solution is slowly added, with stirring, to 1 ml of glucan solution. At 30 seconds, the pH is raised to approximately 9.5 with about 100 μl of 0.2 M TEA (triethylamine) and maintained at about pH 9.5 with TEA for a total of 2.5 minutes to activate the polysaccharide. After 2.5 minutes, 10 mg of the GTF solution is added to the activated glucan, while mixing. The pH should be in the range of 9-9.5. After 2 hours at RT, the reaction is quenched by the addition of 0.5 ml 2 M glycine @ pH 8 and incubated overnight at RT. The conjugate is then dialyzed against PBS, pooled, and sterile filtered with an 0.2 μm Millipore GV filter. Protein concentration in the dialysate is determined using the BCA assay (Pierce Chemical). The concentration of polysaccharides is determined using the resorcinol/sulfuric

acid assay method of Monsigny *et al.*, Anal.Chem., Vol. 175, p. 525 (1988), incorporated herein by reference.

Coupling efficiency using the CDAP procedure is often on the order greater than 70%. Further, when the protein to be coupled tends to bind to the polysaccharide, as with glucan and a GTF, coupling efficiencies will usually exceed 90%. Moreover, unconjugated protein is unlikely to interfere with the antigenicity of the resultant vaccine and may even contribute to the immune response, as suggested by the "Free Protein" application of Lees and Mond (U.S. Serial No. 9,003,155, filed Jan. 6, 1998), incorporated herein by reference. Nevertheless, unconjugated proteins may be removed, for example, by passage over an S500HR (Pharmacia) gel filtration column.

CDAP Conjugation of Peptide Moieties to Glucan, TD Antigens or Glucan-conjugates:

Peptide moieties may be coupled to the protein, polysaccharide, or protein-polysaccharide conjugate using the following general procedure. A peptide is prepared with an amino-terminal cysteine. The cysteine is reduced in 100mM DTT and dialyzed extensively against 10mM sodium acetate, 2mM EDTA, pH 5 at 4°C using a dialysis membrane with a molecular weight cutoff of 500. The thiol content of the peptide is determined using Ellman's reagent. (Ellman, Archiv. Biochem. Biophys. 82:70-77 (1959) (Incorporated by reference).

The protein, polysaccharide, or polysaccharide-protein conjugate to be coupled is dialyzed against 150mM HEPES buffer, 2mM EDTA, pH 7.3 and labeled in the dark with a 20-fold excess of N-hydroxysuccinimide iodoacetate (SIA) (Pierce Chemical) for 2 hours at RT. Unreacted reagents are then removed by overnight dialysis at 4°C using dialysis membrane with a molecular weight cutoff of about 10,000.

The reduced peptide and the protein-SIA are degassed under nitrogen and combined at a molar ration of 1 thiolated peptide/mole SIA at pH 7.3. The coupling reaction is allowed to proceed overnight, under nitrogen, in the dark. The reaction is then quenched by addition of Mercaptoethanol to 0.2mM and unconjugated peptide is removed by ultrafiltration on a membrane with a 50 kDa cutoff and washed into PBS. The peptide and protein content are determined by amino acid analysis and the product is sterile filtered with an 0.2 μm Millipore GV filter.

The conjugates and other reagents used, or to be used, in the following Examples are produced using the general technique described above. Tt-Dex refers to tetanus toxoid conjugated to high molecular weight dextran T2000, (Pharmacia) as described for example, in U.S. Patent 5,585,100, incorporated by reference herein. Tt-WSG and Tt-WIG refer to tetanus toxoid conjugated to water soluble and water insoluble glucan, respectively.

CDAP Conjugation of Tetanus Toxoid to WSG (Tt-WSG)

WSG was suspended in water @ 10 mg/ml on ice, CDAP @ 100 mg/ml in acetonitrile, tetanus toxoid was 16.8 mg/ml in saline (obtained from SmithKline Beecham). At time zero, 18 μl of CDAP solution was slowly added, with stirring, to 0.25 ml of the WSG glucan solution at RT. At 30 seconds, the pH was raised with 18 μl of 0.2 M TEA (triethylamine). At 60 seconds, an additional 9 μl of TEA was added. At 2.5 minutes, a solution of 150 μl of tetanus toxoid and 25 μl of 100 mM sodium borate, pH 9.3, were added with stirring. The coupling reaction was allowed to proceed for 30 minutes at RT, and then quenched by addition of 100 μl of 1M glycine, pH 9.3. Unconjugated Tt was removed by gel filtration on a 1X60 cm S400HR column (Pharmacia), equilibrated in PBS. The void volume fractions containing the Tt-WSG conjugate were pooled and sterile filtered with an 0.2

μm Millipore GV filter. Protein concentration in the pool was determined using the Coomassie Plus assay (Pierce Chemical). The concentration of polysaccharides was determined using the resorcinol assay of Monsigny *et al.*, Anal.Chem., Vol. 175, p. 525 (1988). The protein/carbohydrate ratio of the Tt-WSG conjugate was 0.7 mgTt / mgWSG.

Tt-mp-WSG was prepared as above except that 160 μl tetanus toxoid (Massachusetts Public Health Laboratories @ 15 mg/ml) and 50 μl of 100mM sodium borate were used. The protein/carbohydrate ratio of the conjugate was determined to be 1mg/ml.

CDAP Conjugation of Tetanus Toxoid to WIG (Tt-WIG)

WIG was suspended in saline @ 10 mg/ml on ice, CDAP @ 100 mg/ml in acetonitrile, tetanus toxoid was 16.8 mg/ml in saline (obtained from SmithKline Beecham).

At time zero, 15 μl of CDAP solution was slowly added, with stirring, to 0.23 ml of the WIG glucan solution at RT. At 30 seconds, the pH was raised with 15 μl of 0.2 M TEA (triethylamine). At 2 minutes, 136 μl of tetanus toxoid and 25 μl of 100 mM sodium borate, pH 9.3, were added with stirring. The coupling reaction was allowed to proceed overnight at RT. The reaction mix was then centrifuged to pellet the conjugate. The pellet washed by resuspension in PBS and centrifuged. The pellet was then resuspended in PBS. Protein was determined using the Coomassie Plus assay (Pierce Chemical), and carbohydrate using the resorcinol assay. The protein/carbohydrate ratio of the Tt-WIG conjugate was 0.17 mgTt / mgWIG.

Tt-mp-WIG is similarly prepared using tetanus toxoid obtained from the Massachusetts Public Health Laboratories.

CDAP Conjugation of Tetanus Toxoid to WSG Using a Spacer (Tt-spWSG)

WSG was derivatized with amines using the following procedure. At time zero, 50 μl of CDAP (100 mg/ml in acetonitrile) was added to 1 ml WSG

@ 10 mg/ml, with stirring, at RT. At 30 seconds,50 μl of 0.2 M TEA was added. At 2.5 minutes, 0.5 ml of 0.5 M hexandiamine in sodium borate (pH 9.3) was added and the mixture was stirred for 1 hour at RT. The WSG was desalted on a 1.5x15 cm P6DG column (BioRad), equilibrated with 0.02% NaN_3 in saline The product was recovered and concentrated on a FILTRON Macrosep 30 concentrator. The derivatized WSG was determined to have 20.4 amines/100 kDa of glucan using the method of Monsigny *et al.*, Anal.Chem., Vol. 175, p. 525 (1988) to determine carbohydrate and the NTBS method of Vidal and Franci, J. Immum. Meth. 86:155-56 (1986) to determine amines.

250 μl of the aminated WSG (12mg/ml) was iodoacetylated by stirring with 100 μl of HE buffer (150 mM HEPES, 1 mM EDTA, pH 7.3) and 40 μl of 100 mM N-hydroxysuccinimide iodoacetate (SIA) in dimethylformamide (DMF) for 2 hours at RT in the dark. The reaction was desalted on a 1x15 cm P6DG column (BioRad), equilibrated with 0.02% NaN_3 in saline, and concentrated on a FILTRON Macrosep 30.

Tetanus toxoid (179 μl @ 16.8 mg/ml from SmithKline Beecham) was stirred with 100 μl of 0.1M sodium borate, pH 9.3, and thiolated with 9 μl of 25 mM Traut's reagent (Pierce Chemical) in water. The reaction was allowed to proceed for about 2 hours at RT, then desalted on a 1x15 cm P6DG column (BioRad), equilibrated with 0.02% NaN_3 in saline. The desalted thiolated tetanus toxoid was concentrated on a FILTRON Macrosep 30.

The thiolated tetanus toxoid was combined with the iodoacetylated WSG in a total volume of 0.75 ml. The pH was raised to 7.5 by addition of 50 μl of HE buffer. The reaction was incubated overnight at 4°C then quenched by making 0.2 mM mercaptoethanol for 1 hour. Iodoacetamide was then added to a final concentration of about 10 mM.

The conjugate was purified on a 1X60 cm Pharmacia S400HR column, equilibrated in PBS. The void volume fractions containing the Tt-spWSG conjugate were pooled and sterile filtered with an 0.2 μm Millipore GV filter. Protein and polysaccharide concentrations were determined using the Coomassie Plus and resorcinol assays, respectively. The protein/carbohydrate ratio of the Tt-spWSG conjugate was 1.7 mg Tt / mg WSG.

CDAP Conjugation of Tetanus Toxoid to WIG Using a Spacer (Tt-spWIG)

WIG was derivatized with amines using the following procedure. At time zero, 70 μl of CDAP (100 mg/ml in acetonitrile) was added to 920 μl WIG @ 10 mg/ml in saline, with stirring, at RT. At 30 seconds, 70 μl of 0.2 M TEA (triethylamine) was added. At 2.5 minutes, 0.5 ml of 0.5 M hexandiamine in sodium borate (pH 9.3) was added and the mixture was stirred for about 2 hours at RT. The aminated WIG was dialyzed extensively against PBS. The dialysate tested positive for amines using the TNBS assay. Vidal and Franci, J. Immum. Meth. 86:155-56 (1986).

1 ml of the aminated WIG (4mg/ml) mixed with 100 μl of 5XHE (0.75 M HEPES, 5mM EDTA, pH 7.3). 100 μl of 100 mM N-hydroxysuccinimide iodoacetate (SIA) in dimethylformamide (DMF) was added and the reaction allowed to proceed for about 2 hours at RT. The product dialyzed overnight against saline to remove the reagent.

238 μl of tetanus toxoid (16.8 mg/ml, SmithKline) was stirred with 50 μl of 0.1M sodium borate, pH 9.3, and thiolated with 2.7 μl of 100 mM Traut's reagent (Pierce Chemical) in water. The reaction was allowed to proceed for about 1 hours at RT, then desalted on a 1x15 cm P6DG column (BioRad), equilibrated with 0.02% NaN_3 in saline. The desalted thiolated tetanus toxoid was concentrated on a FILTRON Macrosep 30.

The thiolated tetanus toxoid was combined with the iodoacetylated WIG. The reaction was incubated overnight at 4°C then quenched by making 0.2 mM mercaptoethanol for 1 hour. The product was pelleted by centrifugation and washed 3-4 times by suspension in 1.5 ml PBS followed by centrifugation. Protein and polysaccharide concentrations were determined using the Coomassie Plus and resorcinol assays, respectively. The protein/carbohydrate ratio of the Tt-spWIG conjugate was 0.33 mgTt / mgWIG.

CDAP Conjugation of Tetanus Toxoid to T2000 Dextran (Tt-Dex)

High molecular weight dextran was prepared by fractionating T2000 Dextran (Pharmacia) on an S400HR column, as described in Lees et al., Vaccine 12:1160-66 (1994). The size-fractionated dextran was suspended in saline @ 10 mg/ml, CDAP @ 100mg/ml in acetonitrile, tetanus toxoid was 16.8 mg/ml in saline (obtained from SmithKline Beecham).

At time zero, 18 μl of CDAP solution was slowly added, with stirring, to 0.23 ml of the dextran solution at RT. At 30 seconds, the pH was raised with 18 μl of 0.2 M TEA. At 1 minute, an additional 9 μl of 0.2 M TEA was added. At 2.5 minutes, 150 μl of tetanus toxoid and 25 μl of 100 mM sodium borate, pH 9.3, were added with stirring. The coupling reaction was allowed to proceed for 30 minutes at RT then quenched by overnight incubation with 100 μl of 2M glycine, pH 8 at 4°C.

Unconjugated Tt was removed by gel filtration on a 1X60 cm S400HR column (Pharmacia), equilibrated in PBS. The void volume fractions containing the Tt-Dex conjugate were pooled and sterile filtered with an 0.2 μm Millipore GV filter. The protein/carbohydrate ratio of the Tt-Dex conjugate was 0.84 mg Tt / mg Dextran T2000.

CDAP Conjugation of GTF to WSG Using a Spacer (GTF-spWSG)

At time zero, 50 μl of CDAP (100 mg/ml in acetonitrile) was added to 1 ml *S. mutans* WSG @ 10 mg/ml, with stirring, at RT. At 30 seconds, 50 μl of 0.2 M TEA was added. At 2.5 minutes, 0.5 ml of 0.5 M hexandiamine in sodium borate (pH 9.3) was added and the mixture was allowed to proceed overnight. The product was desalted on a 1.5x15 cm P6DG column (BioRad), equilibrated with 0.02% NaN_3 in saline The product was then recovered and concentrated on a FILTRON Macrosep 30 microconcentrator concentrator. The derivatized WSG was determined to have 68 amines/100 kDa of glucan.

1 ml of the aminated WSG (4.8 mg/ml) was mixed with 100 μl of 5XHE buffer. 100 μl of 100 mM N-hydroxysuccinimide iodoacetate (SIA) in dimethylformamide (DMF) was added and the reaction allowed to proceed for about 2 hours at RT. The product dialyzed overnight against saline to remove the reagent.

GTF in 6M guanidine HCl (at about 0.5 mg/ml) was dialyzed exhaustively against PBS and concentrated using a Filtron Macrosep50. Concentration was estimated as 1.7 mg/ml from the area of the UV peak using SEC HPLC. 1.3 ml of the GTF solution was stirred with 200 μl of 0.1M sodium borate, pH 9.3, and thiolated with 30.6 μl of 10 mM Traut's reagent (Pierce Chemical) in water. The reaction was allowed to proceed for about 2 hours at RT, then dialyzed overnight against PBS to remove the reagents.

The thiolated GTF was combined with the iodoacetylated WIG to a final volume of about 3 ml. The reaction was incubated overnight at 4°C then quenched by making 0.2 mM mercaptoethanol for 1 hour, then made 10 mM in iodoacetamide. The GTF conjugate was dialyzed against PBS and sterile filtered with an 0.2 μm Millipore GV filter. Protein and polysaccharide concentrations were determined using the Pierce Coomassie Plus and

resorcinol assays, respectively. The protein/carbohydrate ratio of the GTF-spWSG conjugate was 0.59 mg GTF / mg WSG.

Example 2

Immunogenicity of Streptococcal Mutans Glucans

Groups of 3 Sprague-Dawley rats were immunized with 1 or 10 μg of WIG, or WSG, or PBS, each incorporated in Freund's adjuvant (DIFCO). Animals were inoculated by subcutaneous injection in the vicinity of the the salivary gland and lymph nodes on day 0 in complete adjuvant, and on day 14 in incomplete adjuvant. Serum and saliva samples were extracted on d14 and d29 and tested for IgG and IgA reactivity with *S. sobrinus* glucan.

The Sprague-Dawley rats used herein are derived from germ-free rats that had been reared in the Area 051 isolator facility of Charles River Laboratories and been found to be free of indigenous mutans streptococci. These rats served as the foundation breeding stock for the dams used in these experiments and are regularly monitored for the absence of mutans streptococci. The mutans-free progeny of the dams are weaned at approximately 21 days and are subsequently fed high-sucrose diet 2000. Taubman and Smith, J. Immunol. 118(2):710-20 (1977).

In a separate experiment, groups of 3 Rowett rats (2-3 month old female animals bred at Forsyth Dental Center) were similarly vaccinated, except that serum and saliva samples were extracted on 14, 25, 35, and 42 days after the primary inocculation.

Levels of IgG and IgA in serum samples were examined by ELISA, as described below. An acceptable ELISA assay is also described in Antibodies: A Laboratory Manual, (Harlow & Lane eds., 1988), Cold Spring Harbor Laboratory Press (incorporated by reference). Anti-glucan titers were highest after immunization with 10 μg of WSG antigen. Maximal salivary IgA antibody to WSG and WIG was observed 25 days after the primary

inoculation. No T-cell proliferative responses against WIG or WSG were observed in cells from glucan-challenged animals.

Example 3

Enhanced Immunogenicity of Glucan Conjugate Vaccines

Gnotobiotic Sprague-Dawley rats were immunized subcutaneously in the salivary gland vicinity with PBS, WIG, WSG, Tt, or the Tt conjugates described in Example 1. All polysaccharide inocula were used at doses of 1 or 10 μg (PS Dose). As controls, 1 or 10 μg of tetanus toxoid was injected into Tt animals as controls. Rats were immunized on day 0 with antigen in complete Freund's adjuvant (CFA) and boosted on d14 with the same dose of antigen suspended in incomplete Freund's adjuvant (IFA). Saliva and blood taken from tail veins are collected on d28 and d42 and analyzed for levels of IgG (blood) and IgA (saliva) reactive with WIG, WSG, and Tt.

The compilation of the serum antibody titer data presented in Table I. The results of additional experiments using WSG-GTF conjugates is presented in Figure 1. In addition, WSG-Tt and WSG-GTF conjugates additionally comprising mutans-derived peptide moities will be prepared.

T cell Proliferation Assay

Stimulation index (SI) was used to measure the T cell proliferative response induced by the various inoculations. Briefly, T cells were isolated from cervical, brachial, and axillary lymph nodes using standard techniques. The cells were exposed to tritiated thymidine in the presence or absence of Tt, Tt-WSG or Tt-WIG. SI index is the ratio of ^{3}H incorporation levels as in the presence/absence of antigen. Each SI index in Table I is mean value from 3 animals. Proliferation assays for each animal are done in triplicate and used to calculate the mean value for the group.

Determination of Antibody Titers:

Antibody titers were determined by a microtiter plate ELISA assay, essentially as described in Stack *et al.*, Oral Microbiol. Immunol. 5:309-14

(1990) and Taubman et al., Infect. and Immun. 63:3088-93 (1995), Cox and Taubman, Molec. Immunol. 19:171-78 (1982); Cox et al., Molec. Immunol. 17:1105-15 (1980); and Engvall and Perlmann, J. Immun. 109:129-35 (1972), incorporated by reference.

The antigens used to coat the wells of 96 well microtiter plates are as follows WSG (10 μg/well), WIG (0.1 μg/well), and Tt (0.1 μg/well). Isotype specific rabbit anti-rat IgA or IgG is used with goat anti-rabbit IgG alkaline phosphatase. (TAGO Inc.) The plates are developed with *p*-nitrophenyl-phosphate (Sigma) and read on a photometric scanner (Dynatech) at 405 nm. Antibody of each isotype (IgG and IgA) is expressed seperately as ELISA units (EU) of a particular isotype, which are calculated relative to the titration of reference sera from Sprague-Dawley rats hyperimmunized with Tt or with intact mutans streptococci grown in sucrose-containing media. Titrations of reference sera are assayed on each ELISA plate along with titrations of serum or saliva samples. Undiluted reference sera is arbitrarily assigned a value of 100 ELISA units. Sample values are compared to the reference values on each plate to determine the EU value of a sample. It is understood that EU values of different animals, or groups of animals, can be compared with respect to a particular ELISA antigen. However, absolute EU values cannot be directly compared between different ELISA antigens. For example, a sample having an EU of 150 against WSG would have 10 times the amount of anti-WSG activity as a different sample containing 15 EU against the same antigen. However, a sample having 15 EU on a WSG assay, and 150 EU on a WIG assay, does not necessarily contain more or even comparable levels of anti-WIG antibodies.

The results in Table I indicate that covalent binding of Tt to WSG or WIG significantly enhances the anti-glucan serum IgG. Moreover, administration of glucan conjugated to Tt can also enhance the level of anti-glucan IgA antibodies in saliva. There is also a significantly enhanced response to Tt in the conjugates as compared to Tt alone. Indeed, even

though the dose of unconjugated Tt in Group 14 is greater than the dose of Tt dose administered as a conjugate in Groups 2, 4, 6, 8, 10, or 12, the antibody responses to Tt are almost invariably higher in the conjugate groups. In addition, the T cell proliferation response to Tt is dramatically higher in animals that received conjugated Tt as compared to animals that received Tt alone. Thus, covalent binding of other TD antigens to the glucan carrier, such as GTF or other mutans proteins, will similarly enhance the antigenicity of the mutans-derived TD antigen and contribute to the prophylactic effect of the vaccine conjugate.

Sera from animals injected with Tt-Dex cross-react with glucan epitopes in the ELISA assay. The basis for this phenomenon is unknown. However, dextran (Dex) is a linear polymer of α1-6 linked glucose, and glucans, especially water soluble glucans, contain stretches of α1-6-linked glucose polymer. Consequently, the observed cross-reactivity may reflect antibodies specific for the linear α1-6 portions of the glucan. Thus, the antibodies elicited by the Tt-Dex conjugate may provide a prophylactic effect against mutans infection which has not been previously investigated.

Table I

		Mean Serum IgG EU Antibody (EU) vs:			Mean Saliva IgA Antibody (EU) vs:			Mean SI vs:		
Group/Ag	PS Dose (μg)	WSG	WIG	Tt	WSG	WIG	Tt	Tt	TtWSG	TtWIG
1. Tt-Dex	1	54	217	10						
2. Tt-Dex	10	31	318	180	90	136	9	4.1		
3. Tt-WSG	1	27	267	20						
4. Tt-WSG	10	266	398	540	90	95	32	8.9	9.1	6.1
5. Tt-WIG	1	<1	6	<1						
6. Tt-WIG	10	3	62	100	10	7	3	5.2		
7. Tt-spWSG	1	15	163	66						
8. Tt-spWSG	10	38	223	300	35	46	11	2.5		
9. Tt-sp-WIG	1	1	21	20						
10. Tt-sp-WIG	10	2	44	210	10	23	14	2.4		
11. Tt-mp-WSG	1	49	150	20						
12. Tt-mp-WSG	10	79	222	110	113	209	23			
13. Tt (1μg)	-	<1	10	<1						
14. Tt (10μg)	-	<1	1	110	17	19	7	1.8	1.8	2.8
15. WSG	1	<1	<1	<1						
16. WSG	10	<1	7	<1	50	78	4	0.7		
17. WIG	1	<1	<1	<1						
18. WIG	10	<1	<1	<1	19	31	5	0.7		
19. PBS	-	<1	<1	<1	15	6	3	1.1	1.1	1.2

Example 4

Glucan Conjugate Vaccines Inhibit WSG synthesis

Groups of 7, mutans-free, male Sprague-Dawley rats of approximately 21-23 days of age were injected subcutaneously in the vicinity of the salivary glands (sgv) on d0 with PBS (sham), or with 1 or 10 μg doses of antigen (WSG, GTF, GTF-WSG, Tetanus toxiod (Tt), or Tt-WSG) in complete

Freund's antigen, as described above. On d7, and again on d117, each animal was boosted with the same dose of antigen or PBS suspended in incomplete Freund's adjuvant (IFA).

Blood samples taken on days 21, 35, 47, 124, 131, 145, 159 and 175 were analyzed for serum IgG titers to WSG and GTF. As shown in Figure 1, immunization with the GTF-WSG conjugate results in a marked increase in serum IgG immune response to WSG. In contrast, immunization with GTF alone produces only a marginal increase in anti-WSG titer.

Sera from immunized rats was analyzed for the ability to inhibit GTF-mediated glucan polymerization. Briefly, GTF activity is measured by determining the extent of ^{14}C-glucose incorporation from gucosyl-labeled sucrose into complex polysaccharides as described in Taubman *et al.*, Infect. Immun. 63:3088-93 (1995) and Taubman and Smith, J. Immunol. 118:710-20, both of which are incorporated by reference. Although inoculation with either GTF or GTF-WSG inhibits the production of WIG from sucrose, WSG synthesis is significantly inhibited only in sera of animals vaccinated with the GTF-WSG conjugate.

On d175, the animals were sacrificed and stimulation indices (SI) determined using the T cell proliferation assay described above. The results of this assay are presented in Table II. T cell proliferation responses were significantly elevated in animals immunized the GTF and GTF-WSG conjugate.

Table II

Immunized Group	Test Antigens Stimulation Index			
	WSG	Tt-WSG	GTF-WSG	GTF
Tt-WSG	1±0	10±2	4±1	1±0
Tt	1±0	4±1	2±1	2±1
GTF-WSG	1±0	1±0	19±1	15±3
GTF	1±0	1±0	20±6	17±5

Example 5

Glucan Conjugate Vaccines Stimulate Production of Salivary Anti-IgA Antibodies Against WSG and GTF

Groups of 10-11, mutans-free, male Sprague-Dawley rats of approximately 40 days of age were injected subcutaneously in the vicinity of the salivary glands (sgv) on d0 with PBS (sham), or 10 μg doses of WSG, GTF, GTF-WSG, Tetanus toxoid (Tt), or Tt-WSG, as described above, each suspended in Freund's complete adjuvant. On d7, each animal was boosted with the same dose of antigen or PBS suspended in incomplete Freund's adjuvant (IFA).

Beginning on day 22, rats were orally infected with approximately 10^8 *S. sobrinus* 6715 cells for 3 consecutive days. Rats were singly caged after the infection series until terminated at d101.

The presence of mutans streptococcal flora was assessed at 32 days and at termination, as described in Taubman *et al.*, Infect. Immun. 63:3088-93 (1995), incorporated by reference. Briefly, teeth were systematically swabbed and the swabs sonicated. The sonicate was serially diluted and plated onto mitis salivarius (MS) agar (to determine total streptococci), and on MS agar further including 200 µg of streptomycin per ml (MSS agar).

Plates were incubated at 37° in 90% N_2--10%Co_2 for 48 hours, at which time total and mutans streptococci CFU were enumerated microscopically.

All intentionally infected rats exhibited significant titers of the *S. sobrinus* at both time points, indicating successful and stable colonization with the test bacteria. To ascertain that no horizontal transmission occurred, sentinal animals housed in close proximity were tested concurrently. As expected, swabs from these control animals were negative for *S. sobrinus*.

Blood and saliva samples taken on d14, d21, d35, d47, and d101 were analyzed for levels of serum IgG and salivary IgA antibodies against WSG and GTF. Higher levels of salivary anti-WSG IgA were induced by the conjugate than by GTF alone (Figure 3A). These levels remained elevated through d101 (Figure 3B). Similarly, salivary IgA antibodies directed against GTF were substantially elevated after GTF-WSG immunization (Figure 4A-C).

Example 6

Glucan Conjugate Vaccines Reduce the Incidence of Dental Caries

The sacrificed animals of Example 5 are examined for caries. The extent and depth of carious lesions in all rat molar teeth are evaluated microscopically using the modified Keyes method described in Taubman and Smith, J. Immunol. 118:710-20 (1977), incorporated by reference. Caries scores are determined separately on smooth and occlusal surfaces.

Comparison of caries scores between controls and animals vaccinated with the compositions of the invention will demonstrate that the claimed compositions elicit protection against dental caries.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification, all of which are hereby incorporated by reference in their entirety. The embodiments within

the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan recognizes that many other embodiments are encompassed by the claimed invention and that it is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

We claim:

1. An immunogenic composition comprising:
   1) at least one streptococcal glucan, and
   2) at least one moiety, directly or indirectly, covalently conjugated to the glucan;

wherein the immunogenic composition elicits antibodies to both the at least one streptococcal glucan and the at least one moiety..

2. The immunogenic composition of claim 1 wherein at least one moiety is a T cell-dependent (TD) antigen.
3. The immunogenic composition of claim 1 wherein the glucan is water soluble glucan (WSG).
4. The immunogenic composition of claim 1 wherein the streptococcal glucan is from *S. mutans*.
5. The immunogenic composition of claim 1 wherein the streptococcal glucan is from *S. sobrinus*.
6. The immunogenic composition of any of claims 1-5 wherein at least one moiety is selected from the group consisting of: glucosyltransferase (GTF), a recombinant GTF fusion protein, and an immunogenic fragment of a GTF.
7. The immunogenic composition of claim 1 wherein at least one moiety induces antibodies that specifically bind to at least one epitope of a mutans streptococcus.
8. The immunogenic composition of claim 7 wherein the antibodies interfere with the catalytic and/or glucan binding activity of a GTF.
9. The immunogenic composition of claim 7 wherein the antibodies interfere with the binding properties of a GBP.
10. The immunogenic composition of claim 7 wherein the antibodies interfere with the binding properties of AgI/II.

11. The immunogenic composition of claim 7 wherein the at least one epitope is selected from the group of peptide sequences consisting of:

a)	DGKLRYYDANSGDQAFNKSV	SEQ ID NO:1;
b)	PLDKRSGLNPLIHNSLVDREVDDRE	SEQ ID NO:2;
c)	TGAQTIKGQKLYFKANGQQVKG	SEQ ID NO:3;
d)	QWNGESEKPYDDHL	SEQ ID NO:4;
e)	GGYEFLLANDVDNSNPVVQ	SEQ ID NO:5;
f)	ANDVDNSNPVVQAEQLNWL	SEQ ID NO:6;
g)	GGYEFLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:7;
h)	GGYEDLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:8;
I)	GGYEFLLANDVDNSNPIVQAEQLNWL	SEQ ID NO:9;
j)	AGYELLLANDVDNSNPVVQAEQLNHL	SEQ ID NO:10;
k)	DANFDSIRVDAVDNVDADVVQIA	SEQ ID NO:11;
l)	DANFDSIRVDAEDNVDADQLQIS	SEQ ID NO:12;
m)	GAVDSILGGVATYGA	(SEQ ID NO:16);
n)	YEKEPTTPPTRTPDQ	(SEQ ID NO:17);
o)	TPEDPTDPTDPQDPSS	(SEQ ID NO:18); and
p)	ANAANEADYQAKLTAYQTEC	(SEQ ID NO:19).

12. The immunogenic composition of claim 2 wherein one or more additional moieties are conjugated to the glucan, to at least one T-dependent antigen, or to both.

13. A method of making the immunogenic composition of claim 1 comprising:

directly or indirectly conjugating the T-dependent antigen to the streptococcal glucan, and

recovering the conjugated product.

14. The method of claim 13 wherein the conjugation method is CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate) activation of the glucan.

15. A method of making antibodies comprising administering to the immunogenic composition of claim 1-5, or 7-11 to a host, and recovering the antibodies.

16. The method of claim 15 wherein the antibodies are monoclonal.

17. The method of claim 16 further comprising the step of humanizing the recovered antibodies.
18. A method of treating a patient comprising administering the antibodies of claim 15 to a patient.
19. A vaccine, comprising:
    an immunostimulatory amount of at least one of the compositions of claims 1-5, or 7-11, dissolved or suspended in a pharmaceutically acceptable carrier or delivery vehicle.
20. A method of treating a patient, comprising administering the vaccine of claim 19 to a patient.
21. The method of claim 20 wherein the patient is a child between the ages of two months and thirty-six months of age.
22. An immunogenic composition comprising:
    1) a Type 2 T cell-independent antigen, and
    2) at least one moiety covalently bound to the T-independent antigen;
    wherein the T-dependent antigen or at least one moiety induces antibodies that specifically bind to at least one protein component of a mutans streptococcus.
23. The immunogenic composition of claim 22 wherein at least one moiety comprises at least one antigenic epitope of a mutans streptococcus.
24. The immunogenic composition of claim 23 wherein at least one moiety is selected from the group consisting of a glucosyltransferase (GTF), a recombinant GTF fusion protein, and an immunogenic fragment of a GTF.
25. The composition of claim 22 wherein the moiety induces antibodies that specifically bind to at least one epitope of a mutans streptococcus.
26. The immunogenic composition of claim 25 wherein the antibodies interfere with the catalytic and/or glucan binding activity of a GTF.

27. The immunogenic composition of claim 25 wherein the antibodies interfere with the binding properties of a GBP.

28. The immunogenic composition of claim 25 wherein the antibodies interfere with the

binding properties of AgI/II.

29. The immunogenic composition of claim 25 wherein the at least one epitope is a peptide selected from the group of peptide sequences consisting of:

a) DGKLRYYDANSGDQAFNKSV	SEQ ID NO:1;
b) PLDKRSGLNPLIHNSLVDREVDDRE	SEQ ID NO:2;
c) TGAQTIKGQKLYFKANGQQVKG	SEQ ID NO:3;
d) QWNGESEKPYDDHL	SEQ ID NO:4;
e) GGYEFLLANDVDNSNPVVQ	SEQ ID NO:5;
f) ANDVDNSNPVVQAEQLNWL	SEQ ID NO:6;
g) GGYEFLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:7;
h) GGYEDLLANDVDNSNPVVQAEQLNWL	SEQ ID NO:8;
I) GGYEFLLANDVDNSNPIVQAEQLNWL	SEQ ID NO:9;
j) AGYELLLANDVDNSNPVVQAEQLNHL	SEQ ID NO:10;
k) DANFDSIRVDAVDNVDADVVQIA	SEQ ID NO:11;
l) DANFDSIRVDAEDNVDADQLQIS	SEQ ID NO:12;
m) GAVDSILGGVATYGA	(SEQ ID NO:16);
n) YEKEPTTPPTRTPDQ	(SEQ ID NO:17);
o) TPEDPTDPTDPQDPSS	(SEQ ID NO:18); and
p) ANAANEADYQAKLTAYQTEC	(SEQ ID NO:19).

30. The immunogenic composition of claim 22 wherein the T-independent antigen is a high molecular weight bacterial polysaccharide.

31. The immunogenic composition of claim 22 wherein the T-independent antigen is a dextran.

32. A vaccine, comprising:

an immunostimulatory amount of at least one of the compositions of claim 22-31, dissolved or suspended in a pharmaceutically acceptable carrier or delivery vehicle.

33. A method of treating a patient comprising administering the vaccine of claim 32 to a patient.
34. The method of claim 33 wherein the patient is a child between the ages of two months and thirty-six months of age.
35. A method of preventing colonization of mutans streptococci in a patient comprising the administration of an immunostimulatory amount of the immunogenic composition of claims 1 or 22.
36. A method of reducing the amount of mutans streptococci in a patient comprising the administration of an immunostimulatory amount of the immunogenic composition of claims 1 or 22.
37. A method of reducing incidence of dental caries in a patient comprising the administration of an immunostimulatory amount of the immunogenic composition of claims 1 or 22.
38. A method for treating a patient which comprises administering an immunostimulatory amount of a composition comprising a glucose polymer to a patient, wherein said polymer contains at least one of α-1,3 or α-1,6 glucoside linkages.
39. The method of claim 38 wherein the patient is a child between the ages of two months and thirty-six months of age.
40. The method of claim 38 wherein administration of an immunostimulatory amount of said composition to a patient prior to colonization with mutans streptococci prevents the colonization of mutans streptococci in the patient.
41. The method of claim 38 wherein administration of an immunostimulatory amount of said composition to a patient reduces the amount of mutans streptococci in the patient.
42. The method of claim 38 wherein administration of an immunostimulatory amount of said composition to a patient reduces the incidence of dental caries in the patient.

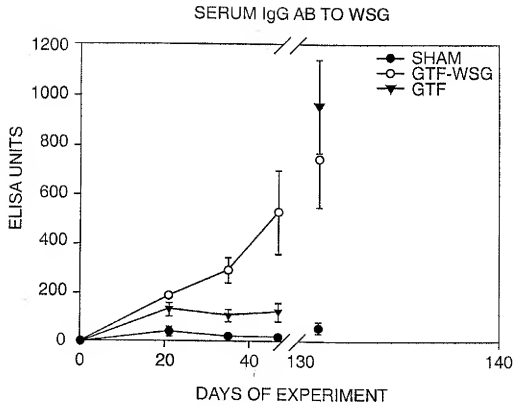
SERUM IgG AB TO WSG
1200
1000
800
600
400
200
0
ELISA UNITS
SHAM
GTF-WSG
GTF
0
20
40
130
140
DAYS OF EXPERIMENT

FIG. 1

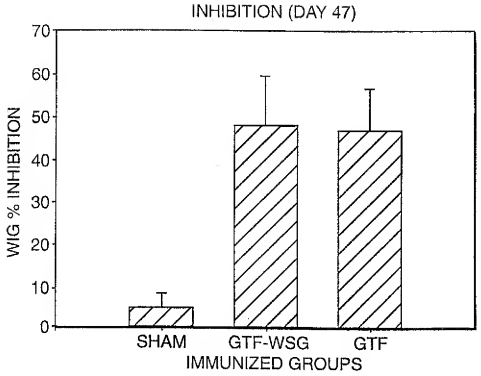
INHIBITION (DAY 47)
WIG % INHIBITION
70
60
50
40
30
20
10
0
SHAM
GTF-WSG
GTF
IMMUNIZED GROUPS

FIG. 2A

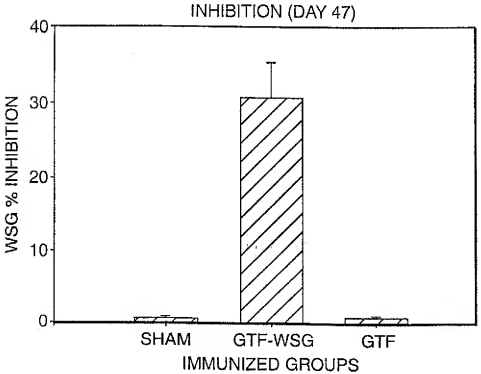
INHIBITION (DAY 47)
WSG % INHIBITION
40
30
20
10
0
SHAM
GTF-WSG
GTF
IMMUNIZED GROUPS

FIG. 2B

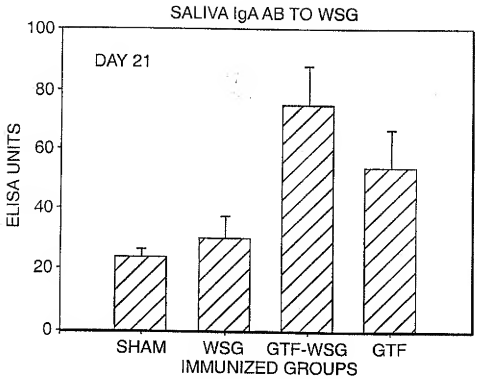
SALIVA IgA AB TO WSG
DAY 21
ELISA UNITS
100
80
60
40
20
0
SHAM
WSG
GTF-WSG
GTF
IMMUNIZED GROUPS

FIG. 3A

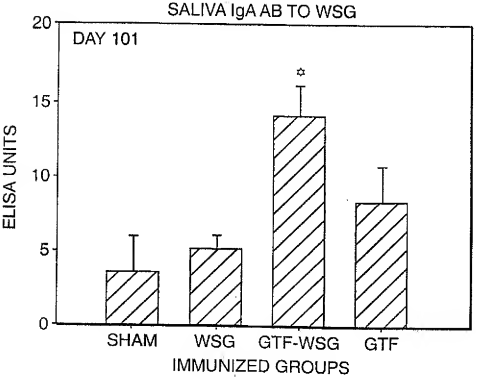
SALIVA IgA AB TO WSG
DAY 101
ELISA UNITS
20
15
10
5
0
SHAM
WSG
GTF-WSG
GTF
IMMUNIZED GROUPS

FIG. 3B

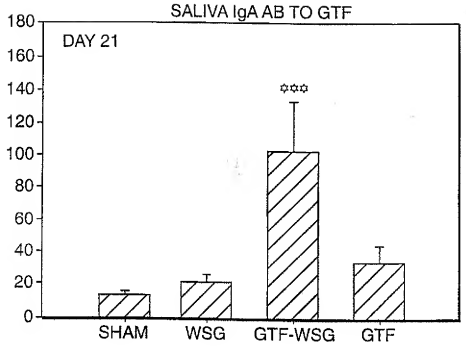
SALIVA IgA AB TO GTF
DAY 21
180
160
140
120
100
80
60
40
20
0
¤¤¤
SHAM
WSG
GTF-WSG
GTF

FIG. 4A

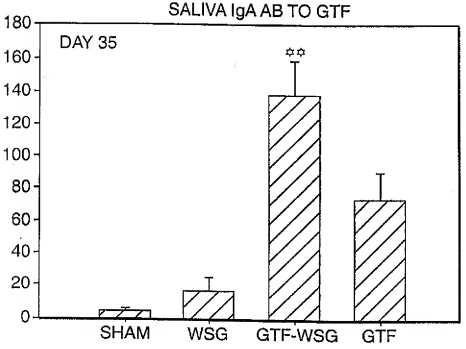
SALIVA IgA AB TO GTF
DAY 35
180
160
140
120
100
80
60
40
20
0
¤¤
SHAM
WSG
GTF-WSG
GTF

FIG. 4B

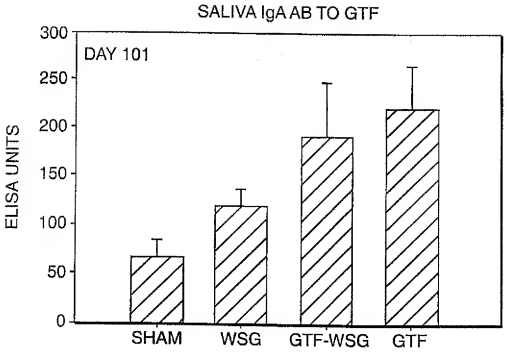
SALIVA IgA AB TO GTF
DAY 101
ELISA UNITS
300
250
200
150
100
50
0
SHAM
WSG
GTF-WSG
GTF

FIG. 4C